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A SIMPLIFIED METHOD TO DETECT EPIDIDYMAL SPERM ANEUPLOIDY (ESA) IN MICE USING THREE-CHROMOSOME FISH

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We developed a new method (ESA) to detect aneuploidy and polyploidy in epididymal sperm of mice using three-chromosome FISH. In comparison to a previous method (TSA-testicular spermatid aneuploidy), which required late-step spermatids, the ESA method utilizes epididymal sperm, which are easier to collect than testicular cells. The ESA method also provides a homogenous population of cells, which significantly speeds up the scoring procedure. A total of 6 mice were investigated by the ESA method and results compared with those obtained by the TSA method: 2 mice each of Robertsonian (8.14) heterozygotes, Rb(8.14) homozygotes and B6C3F1. About 10,000 sperm were scored per mouse. For the ESA method, epididimides were cut into small pieces and filtered. Sperm were prepared for hybridization by sonication and a modification of the DTT/LIS method previously described. Sperm aneuploidy was detected by multi-color FISH using three DNA probes specific for mouse chromosomes X, Y and 8. The sex ratio of X8(49.7%) and Y8(49.6%) did not differ from the expected 1:1. The efficiency of ESA was very high; ~0.3% of the cells showed no hybridization domain. Hyperhaploidy frequencies for chromosomes X, Y and 8 compared well between the ESA and TSA methods for Rb(8.14) heterozygous ($p=0.79$) and B6C3F1 mice ($p>0.05$). The data obtained from Rb(8.14) homozygotes were similar to those from B6C3F1, as predicted ($p=0.3$). This highly efficient ESA assay is, therefore, recommended for future studies of the mechanism of induction of aneuploidy in male germ cells. It also lays a solid foundation for automated scoring. [Work was performed under the auspices of the U.S. DOE by the Lawrence Livermore National Lab. under contract W-7405-ENG-48; with support from NIEHS Y01-ES-10203-00].